CARBOHYDRATE METABOLISM IN ALCOHOL-INDUCED FATTY LIVER

Evidence for an abnormal insulin response to glucagon in alcoholic liver disease

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An intravenous glucose tolerance test and an intravenous glucagon test were performed in 21 alcoholics with fatty liver. The effect on insulin secretion and blood glucose concentrations was compared with the effect in 21 normal subjects and 21 patients, of the same age and weight, with alcoholic cirrhosis. The average glucose tolerance in patients with fatty liver was lower than normal. Forty-eight per cent had a diabetic glucose tolerance. The glucose tolerance in cirrhosis was significantly lower, and it was impaired in 86% of the patients with alcoholic cirrhosis. In both cirrhosis and fatty liver the concentrations of insulin were above normal before and during the two tests. The insulin-glucose ratio was increased in the liver patients during glucagon stimulation, and there was no significant difference between the two types of liver disease. During glucose stimulation the insulin-glucose ratios were equal in liver patients and normal subjects. It is concluded that the carbohydrate metabolism in alcoholic fatty liver is impaired as in alcoholic cirrhosis, although to a lesser degree. The abnormal response to glucagon is suggested to be due to an increased β-cell sensitivity in fasting chronic alcoholics.

The stages by which alcoholic liver injury proceeds in man is not established, particularly not the degree to which steatosis contributes to the transition to cirrhosis. 1

It is now widely recognized that cirrhosis is often accompanied by a decreased diabetic glucose tolerance and hypersecretion of insulin. 2-8

In this study the degree of carbohydrate disturbances in ethanol-induced fatty liver was studied by intravenous injections of glucose and glucagon in subjects with alcoholic steatosis of the liver. The effect on insulin secretion was compared with that found in normal subjects and in patients with alcoholic cirrhosis.

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Experimental Procedures

Patients and control subjects. Three groups, each consisting of 21 patients, were investigated: those with fatty liver, cirrhosis control subjects, and normal control subjects. The sex distribution, age, height, and weight are shown in table 1.

The patients with fatty liver had all been chronic alcoholics (i.e., > 60 g of ethanol per day) for more than 5 years, regularly controlled in an outpatient clinic. Their liver disease was followed by liver biopsies once a year. At the time of investigation their steatosis was classified as moderate or severe.

All of the patients with cirrhosis, defined by necrosis, fibrosis, and nodular parenchymal regeneration, were chronic alcoholics and followed by biopsies once a year. Histologically the cirrhosis was characterized as micronodular with fatty infiltration in all patients.

None of the patients in the two alcoholic groups had alcoholic hepatitis, nor were they treated with steroids or diuretics. No alcohol was consumed for at least 12 hr before the investigation.

None of the normal control subjects had recognized diabetes mellitus, liver disease, or known abuse of alcohol. Only persons with a normal K value (i.e., > 1.05) from the intravenous glucose tolerance test, and with normal concentration of serum glutamic pyruvate dehydrogenase, were encountered in the normal control group.

Methods. All subjects were on a carbohydrate-rich diet in the days before and between the investigations. An intravenous glucose tolerance test (IVGTT) was performed in all persons by injecting 50 ml of 50% glucose over a period of 2 min. Eighteen of the patients with steatosis, 13 of the healthy control subjects, and 13 cirrhotic patients were tested by an intravenous injection of 1 mg of monocomponent pancreatic pork glucagon (NOVO) containing less than 0.002 per thousand of insulin, injected over a few seconds.

The tests were performed in the morning after 10 to 12 hr of fasting. There were at least 3 days between the IVGTT and the glucagon test. The subjects were supine during the tests. A Stille infusion cannula was inserted in the antecubital veins on both sides; one side for injection, the other for blood sampling. In order to avoid clotting 0.9% sodium chloride was filled in the cannulas between drawings of blood samples. The first portion of each sample was discarded. Blood was drawn before and 5, 10 20, 30, 40, 50, and 60 min after injection of either glucose or glucagon.

Laboratory investigations and calculations. Blood glucose concentrations were measured by a glucose oxidase method* on a Technicon AutoAnalyzer. The levels of immunoreactive insulin in serum were measured by the radioimmunoassay of Ørskov. The sensitivity in this laboratory is < 1 μU of immunoreactive insulin per milliliter of serum. The precision expressed as the coefficient of variation is 1 to 2% in the range from 5 to 200 μU of immunoreactive insulin per milliliter of serum. The insulin antiserum employed binds both pro-insulin and insulin. Human monocomponent insulin (NOVO) was used as a standard (1 mg = 25 international units, mol wt 5,804).

The glucose tolerance was evaluated from the K value [K = ln 2 × (100/tₗ)] of the IVGTT according to Lundbæk.

To evaluate if differences in insulin secretion were due to differences in blood glucose levels only, the insulin secretion is expressed relatively to the blood glucose alterations as the insulinoenic index; Δ insulin/Δ glucose. In this study, Δ means the area under the blood glucose and the serum insulin curve in the time intervals indicated using fasting levels as base line.

In the statistical analysis the Mann-Whitney’s nonparametric U-test with a power efficiency of 95% was used. The type II error (2α) was resolved to 0.05.

Results

Glucose tolerance. The K value from the IVGTT in each subject in the three groups is shown in figure 1 with indication of the medians. The mean of the K values in the control subjects was 1.80 ± 0.10 (SEM), in the steatosis group 1.10 ± 0.09 (SEM), and in the cirrhosis group 0.74 ±
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FIG. 1. K values calculated from intravenous glucose tolerance tests in 21 patients with alcoholic fatty liver, 21 healthy control subjects, and 21 control subjects with alcoholic cirrhosis.

0.05 (SEM). The three groups were significantly different at the 1% level. The K values in the fatty liver ranged from 0.51 to 2.05, and 11 of 21 patients (52%) had a normal glucose tolerance. Among the cirrhotic patients, only 3 out of 21 (14%) had a normal K value. The range was narrower: from 0.41 to 1.16.

Insulin secretion during the intravenous glucose tolerance test. Mean values ± standard error of the mean for serum insulin concentrations during the IVGTT are shown for each of the groups in figure 2. The patients with cirrhosis and those with fatty liver have an equal degree of insulin hypersecretion during the whole IVGTT. The insulin concentrations in the fasting liver patient were increased, mean concentration being highest in fatty liver patients. When the insulin concentrations were related to the glucose concentration, i.e., in the insulinogenic index calculated for the total test period (table 2), and for the individual sampling periods (fig. 3), the insulin increment per glucose increment was normal in the liver patients.

Glycogenolysis induced by glucagon. Figure 4 shows the increase in blood glucose concentration after an intravenous injection of glucagon. The normal control subjects have a greater glycogenolysis than liver patients (P < 0.05), estimated from the difference in areas under the blood glucose curves using fasting level as base line (table 2). The glycogenolyses were similar in steatosis and cirrhosis, although the blood glucose concentrations were increased in cirrhosis.

Insulin secretion induced by glucagon. As shown in figure 5, the average insulin concentration was significantly increased in patients with fatty liver and in cirrhotic

Serum Insulin
µU per ml.

FIG. 2. Serum insulin concentrations (mean ± SEM) in 21 patients with alcoholic fatty liver (Δ—Δ), 21 healthy subjects (●—●), and 21 subjects with alcoholic cirrhosis (○—○) during intravenous glucose tolerance test.
TABLE 2. Mean values of insulinogenic index in patients with alcoholic fatty liver, alcoholic cirrhosis, and normal subjects after intravenous glucose and glucagon injections

<table>
<thead>
<tr>
<th></th>
<th>Glucose (25 g intravenously)</th>
<th>Glucagon (1 mg intravenously)</th>
<th>Glucagon (1 mg intravenously)</th>
</tr>
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<td>5-10</td>
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<tr>
<td>Glucagon Difference</td>
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<tr>
<td>Total</td>
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<tr>
<td>Difference</td>
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</tbody>
</table>

*NS, not significant; i.e., $P > 0.05$.

FIG. 3. Insulinogenic index (mean) in patients with alcoholic fatty liver, alcoholic cirrhosis, and normal subjects during intravenous glucagon and glucose test.

FIG. 4. Blood glucose concentrations (mean ± SEM) in 18 patients with alcoholic fatty liver (Δ——Δ), 13 healthy control subjects (●——●), and 13 subjects with alcoholic cirrhosis (○——○) during intravenous glucagon test.

When correction is made for the insulin secretion induced by glucose after the injection of glucagon, as expressed in the insulinogenic index (table 2), the differences between normal control subjects and liver patients is significant ($P < 0.01$ and $P < 0.05$). As shown in figure 3, the difference in insulinogenic
indices was marked only during the first 30 min of the glucagon test.

Discussion

Carbohydrate metabolism in fatty liver. While the glucose tolerance in fatty liver, although decreased, was significantly higher than in cirrhosis, the hyperinsulinemia during stimulation was almost similar in the two groups of alcoholic liver disease. The results support the idea that fatty liver is a step toward cirrhosis in the evolution of alcoholic liver disease, if the theory of Megyesi et al.\textsuperscript{2} is correct. They postulated that cirrhosis produces endogenous insulin resistance and hyperinsulinism before the glucose tolerance is impaired. According to this theory, this study indicates that insulin resistance is a very early phenomenon in chronic alcoholism. The resistance may initiate before morphological changes in the liver are notable, since fatty liver patients display a fully developed hyperinsulinism and a significantly impaired glucose tolerance.

The pathogenesis of the insulin resistance in chronic alcoholic liver disease still remains obscure. It has been suggested that free fatty acids in elevated concentrations might act as an insulin antagonist.\textsuperscript{13} Recent studies,\textsuperscript{2, 3} however, have failed to support this suggestion, as elevated concentrations of free fatty acids were only found in liver patients with symptomatic diabetes mellitus. Presumably it is a secondary phenomenon. The results on growth hormone abnormalities as a source for insulin resistance in chronic liver disease are at present rather conflicting,\textsuperscript{3, 5} and the correlation of growth hormone elevations to insulin resistance is poor.\textsuperscript{3} The same applies to potassium depletion and steroid metabolism as a cause of insulin resistance.\textsuperscript{3}

An explanation on the insulin resistance and hyperinsulinism might be that the \( \beta \)-cells release a pathological amount of proinsulin. Accordingly, the hyperinsulinism would be an artifact due to a lack of specificity in the insulin assay. To our knowledge this possibility has not yet been investigated.

![Graph showing serum insulin concentrations](image)

**Fig. 5.** Serum insulin concentrations (mean ± SEM) in 18 patients with alcoholic fatty liver (\(\Delta\longrightarrow\Delta\)), 13 healthy control subjects (\(\bullet\longrightarrow\bullet\)), and 13 subjects with alcoholic cirrhosis (\(O\longrightarrow O\)) during intravenous glucagon test.

Insulin response to glucose and glucagon in alcoholic liver disease. Both basal and glucose-stimulated insulin levels appeared elevated in liver patients in this investigation. This is in accordance with many studies on cirrhotics,\textsuperscript{2-4, 6} and with the single report\textsuperscript{14} on insulin levels in 6 fatty liver patients. The fact that the basal and glucose-stimulated insulin concentrations were more enhanced in steatosis is in keeping with the theory of Megyesi et al.,\textsuperscript{2} reflecting a decompensated \( \beta \)-cell function in cirrhosis. The hyperinsulinemia during glucose stimulation seemed closely linked to the blood glucose levels. Hence, the insulinogenic index was normal in both steatosis and cirrhosis during the whole IVGTT. During the glucagon test, the insulinogenic index was abnormally in-
creased in the liver patients. This increment was most pronounced during the first 10 min after glucagon administration. It was not just due to reduced glucose turnover, since the insulin concentrations in the liver patients rose significantly beyond those of the normal subjects. This study thus confirms that glucagon acts directly on the \( \beta \)-cells.\(^{15,16} \)

The increased insulin response to glucagon may be a result of two different mechanisms. The \( \beta \)-cell sensitivity to glucagon may be increased, and/or the elimination of glucagon may be reduced as a result of the liver damage. The latter of these mechanisms is less likely for the following reasons: (1) The abnormal insulin response occurred in the first few minutes after glucagon (figs. 4 and 5), after which the insulin concentrations showed a rapid decrease towards normal concentrations. (2) This pattern was most pronounced in cirrhosis. (3) Other peptide hormones such as insulin\(^5\) and gastrin (unpublished observations) have a normal elimination time in chronic liver disease. The increase in the \( \beta \)-cell sensitivity to glucagon in alcoholic liver disease is thus the most attractive explanation.

The effect of glucagon on the insulin secretion is most likely promoted by activation of the adenyl cyclase and hence cyclic adenosine monophosphate in the \( \beta \)-cell.\(^{17} \) The data, however, do not allow further suggestions on the effect of glucagon on the cyclic adenosine monophosphate system in alcoholic liver disease.

It was recently reported that ethanol in acute experiments primed the glucose-induced insulin secretion,\(^{18} \) but inhibited the \( \beta \) cytotrophic effect of glucagon.\(^{19} \) These observations may well be in agreement with this study on chronic alcoholics. If the insulinotropic effect of glucagon is inhibited daily by alcohol, the glucagon secretion increases as reported by Megyesi et al.\(^2 \) in order to meet the peripheral insulin resistance. In fasting alcoholics administration of pharmacological doses of glucagon would then bring about an abnormally high insulin response, because the usual ethanol blockade is taken away. This attempt at an explanation needs confirmation, since results on both the acute effect of alcohol on insulin,\(^{20,21} \) and the effect of glucagon in cirrhosis\(^4 \) still is conflicting.

**REFERENCES**